Docket No.: 2870-0486PUS1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Fumihide NISHIO

Application No.: 10/501,671 Confirmation No.: 9947

Filed: June 28, 2005 Art Unit: 1651

For: HIGH-CONCENTRATION PREPARATION OF SOLUBLE THROMBOMODULIN Examiner: S. R. Macauley

DECLARATION UNDER 37 CFR § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Fumihide Nishio, declare the following.

I am the inventor of the invention as described and claimed in the above-identified patent application.

I graduated from the Department of Pharmacy at Meijyo University in March of 1989. I then graduated from a Master's course at the Graduate School of Meijyo University, specializing in industrial pharmacy, in March of 1991.

I have been employed by Asahi Kasei Pharma Corporation (formerly an employee of Pharmaceutical Research Laboratory of Toyo Jyozo Co., Ltd., which was merged to be Asahi Kasei Pharma Corporation) since April of 1991. From April 1991 to March 1999, I engaged in the research of oral pharmaceutical preparations. Since April 1999, I have been engaged in the research of injectable pharmaceutical preparations.

I have conducted the following experiments, together with experimental assistants under my direct supervision. Test procedures and results are shown below.

Experiments

1. Materials and Methods

Each pharmaceutical preparation shown in Table 1 was prepared according to the preparation method described below. Sample No. 1 was prepared according to the present invention. Sample Nos. 2-3 are comparative examples.

Table 1

Ingredients	Sample No.		
	No. 1	No. 2	No. 3
Soluble Thrombomodulin (TMD123H) Asahi Kasei Pharma Corporation	10 mg	10 mg	5 mg
Sodium L-Glutamate monohydrate (Special Grade) Wako Pure Chemical Industries, Ltd.	10 mg	10 mg	10 mg
D(-)-Mannitol (Special Grade) Wako Pure Chemical Industries, Ltd.	10 mg	10 mg	10 mg
Polysorbate 80 Nikko Chemicals Co., Ltd.	0.1 mg	-	-

In the experiments, the following materials were used:

(i) Vials: Glass vials without silicone coating, 18 x 33 mm, inside diameter at the top: 9.1 mm, Fuji Glass Co., Ltd.,

(ii) Rubber stopper: V5-F8W, Daikyo Seiko, Ltd., and

(iii) 16AP Cap: Ishida Press Industries.

Preparation Method

Sample No. 1

Preparation of Additive Solution

(1) In a 100 mL measuring flask, Polysorbate 80 (500 mg) was weighed and placed, and then water for injection was added and dissolved, followed by adjusting to 100 mL in total to obtain Solution (1).

(2) In a 20 mL measuring flask, 500 mg of sodium L-glutamate monohydrate and 500 mg of D-(-)-mannitol were weighed and placed, and then 1 ml of Solution (1) was added, followed by adding water for injection for dissolving and adjusting to 20 mL in total.

Preparation of Sample Solution

4 mL of the above additive solution was placed in a 15 mL assist tube. Then 1.6 mL of TMD123H (concentration of soluble thrombomodulin: 62.5 mg/mL) and water for injection were added up to 10 mL and then mixed and stirred. The resulting sample solution was subjected to sterilization by filtration through a filter of 0.22 μm in pore size (MILLEX-GV, Millipore) using a 25 mL disposal syringe (Terumo Corp.), followed by dividing into each 1 mL aliquot in a sterile vial without silicone coating.

Freeze-drying

A freeze-drying step was conducted under the conditions described below in the order of: plugging by half with a rubber stopper → freeze-drying → nitrogen-filling → plugging with the rubber stopper and winding a cap up.

Freeze-drying Conditions

Preliminary cooling (decreasing from room temperature to 15°C in 15 minutes) → main cooling (decreasing from 15°C to -45°C in 2 hours) → retaining (-45°C for 2 hours) → vacuum-starting (-45°C for 18 hours) → warming-up (increasing from -45°C to 25°C in 20 hours) → retaining (25°C for 15 hours) → warming-up (increasing from 25°C to 45°C in 1 hour) → retaining (45°C for 5 hours) → lowering to room temperature (from 45°C to 25°C in 2 hours) → nitrogen-filling for recovering pressure (recovering pressure up to -100 mmHg with nitrogen) → plugging → winding a cap up.

Sample Nos. 2 and 3 were obtained in the same manner as the above method.

Method for Evaluation

An injection syringe (for tuberculin, 1 mL, an injection needle: 26G 0.45 x 13 mm, Terumo Corp.), after suction of 1 mL of distilled water for injection, was inserted into the center of a rubber stopper of a freeze-dried preparation as a sample for evaluation. Then distilled water for injection was injected into the center of a freeze-dried product at a rate

of 0.1 mL/second. After removal of the injection syringe, the mixture was left to stand, and then, the appearance was recorded by photograph 30 seconds after the completion of the injection.

2. Results and Discussion

Figure 1 shows the appearance of Sample No. 1 at the dissolving (10 mg/mL of thrombomodulin in the presence of a surfactant). Figures 2 and 3 show the appearances of Sample Nos. 2 (10 mg/mL of thrombomodulin in the absence of a surfactant) and 3 (5 mg/mL of thrombomodulin in the absence of a surfactant), respectively.

Sample No. 1 (Fig. 1) according to the present invention gave a transparent solution, and from this result, it can be clearly understood that a problem of cloudiness during the dissolution process was completely eliminated. In contrast, Sample No. 2 for comparison (Fig. 2), which contained the same concentration of soluble thrombomodulin as that of Sample No. 1 but without a surfactant, caused a problem of cloudiness during the dissolution process. By comparison of these results, it can be clearly understood that the present invention exhibits the advantageous effect of prevention of cloudiness at the dissolving process.

Further, substantially no problem of cloudiness was observed in Sample No. 3 (Fig. 3) containing the low concentration of soluble thrombomodulin at 5 mg/mL, although the color of the solution was observed to be slightly different from that of Sample No. 1. This result indicates that, in Sample No. 3, the problem of cloudiness at the dissolution of the freeze-dried preparation is not serious and need not be solved.

When a lyophilized preparation is dissolved to obtain a solution for injection, microbubbles are generated in the comparative example, not containing a surfactant, during the dissolution process to obtain a solution at a high TM concentration, i.e., as high as 10 mg/mL or more. Due to this problem, some period of time is required before a clear injectable solution is obtained, which delays the start of administration by injection. In contrast, by using the lyophilized preparation containing a surfactant according to the present invention, rapid preparation of a clear TM solution at a concentration of 10 mg/mL or higher is achievable so that administration can be promptly conducted without delay.

Therefore, I conclude that the present invention has the remarkable advantageous effect that cannot be expected from the prior art.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S. Code 1001 and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

By: Fumihide Nishio Date: Mar. 10, 2010
Fumihide Nishio

Attachments: Figures 1-3